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Acceptability and suitability of eggs of false codling moth (Lepidoptera: Tortricidae) from irradiated parents to parasitism by *Trichogrammatoidea cryptophlebiae* (Hymenoptera: Trichogrammatidae)

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Abstract

We determined the acceptability and suitability of eggs of *Cryptophlebia leucotreta* (Meyrick) to parasitization by *Trichogrammatoidea cryptophlebiae* Nagaraja under no choice and choice situations. Male and female moths were treated (*T*) with 150 or 200 Gy of γ-radiation, inbred or out-crossed to normal untreated (*N*) counterparts, and eggs laid by different crosses were offered to *T. cryptophlebiae* as host material. Newly laid (24-h-old) eggs, as well as eggs that were 48- and 72-h-old were evaluated. In general, all egg treatments in the no choice experiments were acceptable for oviposition and suitable for parasitoid development. However, significant differences in the number of parasitized eggs were detected when one member of the host cross, particularly the female, was treated with γ-radiation or when the host egg age was greater than 24 h. No significant differences were detected in any of the choice experiments. Our results suggest that *T. cryptophlebiae* would accept, successfully develop in, and emerge from FCM eggs laid by the different crosses that would theoretically be present in the field under a sterile insect release program for false codling moth (*N*♀ × *T*♂, *T*♀ × *N*♂, and *T*♀ × *T*♂) and suggest that further evaluations combining releases of irradiated moths and parasitoids are warranted.
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Keywords: *Cryptophlebia leucotreta*; Egg parasitoid; Augmentative biological control; γ-Radiation; Sterile insect technique

1. Introduction

The false codling moth (FCM), *Cryptophlebia leucotreta* (Meyrick), is indigenous to Southern Africa and the Ethiopian region (Catling and Aschenborn, 1974; Stofberg, 1954) and also occurs on the islands of Madagascar, Mauritius, Reunion, and St. Helena (CIBC, 1984). It was first reported as a pest of citrus (*Citrus sinensis* (L.)) in 1899, and it is now considered the key pest of virtually all cultivars of citrus in Southern Africa (Stofberg, 1954), as well as a serious pest of cotton (*Gossypium hirsutum* L.) and maize (*Zea mays* L.) in tropical Africa (Angelini and Labonne, 1970; Reed, 1974). Accidental introduction of FCM is one of the "worst of the worst" threats to United States agriculture (ESA, 2003) and port inspectors have reported intercepting larvae of FCM from a variety of African imports, including citrus, maize, eggplant, cayenne pepper, cola nuts, and cassava (W. Bailey, USDA-APHIS, personal communication).

In South Africa, FCM has four to six non-discrete generations per year (Georgala, 1969; Stofberg, 1954). Females lay individual eggs (100–250/female) on fruit or foliage (Catling and Aschenborn, 1974; Daiber, 1978), and neonate larvae penetrate the fruit where larval development is completed. Mature larvae leave the fruit and spin cocoons near the soil or in bark

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crevices (Georgala, 1969; Stofberg, 1954). FCM has developed resistance to the pesticides commonly used for its control (Hofmeyr and Pringle, 1998) and other control strategies, such as orchard sanitation and the use of pathogens, predators, and parasitoids, have had limited success and cannot be used as stand-alone tactics (Newton, 1998). A sex pheromone has been identified for this species (Henderson and Warren, 1970; Persoons et al., 1976; Read et al., 1968, 1974), however, mating disruption is not used for population suppression.

Currently, an augmentative biological control program using the egg parasitoid *Trichogrammatoidea cryptophlebiae* Nagaraja (Hymenoptera: Trichogrammatidae) is underway in South Africa (Newton, 1989, 1998; Newton and Odendaal, 1990). The parasitoids are mass-reared on FCM eggs and production per month is sufficient to treat 600–800 ha of commercial citrus (S. Honiball, Cederberg Biocontrol Systems, personal communication). However, augmentative releases of *T. cryptophlebiae* cannot by themselves realize the level of control needed in citrus and pesticide applications continue to be used (Newton, 1988).

We are conducting research to develop a sterile insect technique program for FCM (Bloem et al., 2003) to be used in combination with releases of *T. cryptophlebiae*. Both theoretical and experimental evidence suggest that combined releases of sterile insects and parasitoids can provide synergistic pest suppression that is more effective than either technique employed separately (Carpenter, 1993, 2000; Knipling, 1992). In sterile insect release programs for Lepidoptera both treated males and females are released into the environment (Bloem and Bloem, 2000; Stewart, 1984). Because all field matings (including those involving treated moths) result in the production of eggs, a potentially large number of host eggs could be present in areas under sterile insect release. For codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), the combined release of sterile insects and *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) egg parasitoids was first suggested by Nagy (1973). Experiments by Bloem et al. (1998) demonstrated that an additive suppressive effect can be realized when sterile moths are released at a 10:1 overflooding ratio (sterile:wild) together with *Trichogramma platneri* Nagarkatti inside field-cages when compared with cages containing wild moths that received sterile moths or parasitoids only. Herein we report the results of laboratory experiments that determined the acceptability and suitability of eggs laid by FCM pairs to parasitization by *T. cryptophlebiae* under no choice and choice situations. The results of these experiments are discussed in the context of enhancement of FCM pest suppression by a combined release of *T. cryptophlebiae* and irradiated FCM in an area-wide sterile insect release program in citrus.

2. Materials and methods

2.1. Test insects

Trichogrammatoidea cryptophlebiae egg parasitoids and FCM used in these experiments were provided by Cederberg Biocontrol Systems located in Citrusdal, South Africa. The colonies have been in continuous culture since 1978, with the stock replaced/replenished with wild FCM at irregular intervals. FCM eggs serve as host material for production of the parasitoids and are reared on an autoclaved maize meal paste inoculated with *Rhizopus* sp. as described by Ripley et al. (1939) and modified by Theron (1947). Adult FCM are collected and placed inside large kitchen sieves on top of waxed paper egg sheets. Egg sheets are removed daily and exposed to parasitoids inside plastic containers for 24 h.

2.2. Egg sheet preparation

2.2.1. No choice experiments

Experiments were conducted in October–November 2002 at the Citrus Research International and INFRUITEC laboratories in Citrusdal and Stellenbosch, South Africa, respectively. Mature FCM pupae were removed from their cocoons, sorted by sex, placed in individual glass vials, and allowed to emerge at $26 \pm 1\,°C$, a 14:10 (L:D) h photoperiod, and 65–70% RH. Cohorts of newly emerged (<24-h-old) virgin adult FCM males and females were chilled (0–2 °C) and exposed to γ-radiation. The irradiator was a panoramic Cobalt-60 point source (currently approximately 6000 Ci) centrally located in a turntable 1 m in diameter. Treatment samples were placed on one or more of eight smaller turntables, each 200 mm in diameter and situated equidistant on the periphery of the main turntable. The smaller turntables counter rotated to enable 360° exposure of the treatment samples. Dose rates measured in sample positions were verified with each exposure, and calculated at 6.27 Gy/min ($\pm <5\%$, Fricke dosimetry). The FCM were treated with doses of 0, 150, and 200 Gy. After irradiation, individual moth pairs were placed inside mesh domes (5-cm diameter $\times$ 2.5-cm high) inverted on top of four waxed paper oviposition sheets (10 cm $\times$ 10 cm) placed on top of styrofoam boards. Insect pins were used to secure the domes on top of the egg sheets. Five replicates were set-up for the following crosses at each dose: N (= untreated) ♀ × T (= treated) ♂, T♀ × N♂, and T♀ × T♂. Moth pairs were allowed to mate and lay eggs on the waxed paper at the above mentioned conditions for 4 days. The first egg sheet was collected after 12 h and discarded. Subsequently, one egg sheet was removed every 24 h for 3 days (to obtain egg sheets that were 72-, 48-, and 24-h-old) and placed inside individual snap-top plastic containers

(5.5-cm diameter × 3.5-cm high). Egg sheets collected on day 1 and 2 were incubated at 26 ± 1 °C, a 14:10 (L:D) h photoperiod, and 65–70% RH for 48 and 24 h, respectively, to allow for egg development. For each FCM cross (*N*♀ × *T*♂, *T*♀ × *N*♂, and *T*♀ × *T*♂), three doses of radiation (0, 150, and 200 Gy) and three host egg ages were prepared (72, 48, and 24 h). On day 5, moths were killed by freezing, and females from each treatment were dissected to determine their mating status (spermatophores present in the bursa copulatrix) (Ferro and Akre, 1975).

2.2.2. Choice experiments

Procedures used in choice experiments were the same as above with the following exceptions. Mesh domes were modified by the addition of a screen divider that allowed placement of two isolated pairs of FCM per dome. A pair of untreated FCM adults was placed in the left side of each dome and a pair of FCM from one of six different treatments was placed in the right side of the dome. Domes were inverted on top of two waxed paper oviposition sheets. In total, five replicates of six different egg treatments (three crosses—*N*♀ × *T*♂, *T*♀ × *N*♂, and *T*♀ × *T*♂- and two doses per cross–150 and 200 Gy) were prepared for exposure to parasitoids. The first egg sheet was collected after 12 h and discarded as above; only eggs laid on the second sheet during the next 24 h period were used.

2.3. Exposure to parasitoids

Parasitized FCM egg sheets obtained from Cederberg Biocontrol Systems were placed inside large glass containers (45-cm high × 20-cm diameter) and kept at 26 ± 1 °C, a 14:10 (L:D) h photoperiod, and 65–70% RH until *T. cryptophlebiae* began emerging. Adult parasitoids were collected from the containers every few hours and placed in plastic petri dishes to ensure that only wasps emerged within 24 h were used in the experiments. Wasps were kept together for 12 h (to ensure mating), after which three female *T. cryptophlebiae* were transferred to each egg sheet container with a fine-tipped brush and allowed to parasitize FCM eggs for 5 h. Females were then removed after 5 h, and egg sheets were incubated at the above conditions for 7 days to allow for complete egg and parasitoid development.

2.3.1. No choice experiments

Exposure of FCM eggs to *T. cryptophlebiae* was done on day 5 after initiation of the experiment (see above). After incubation, mean number of parasitized eggs, the mean number of parasitized eggs from which one or more parasitoids emerged, the mean number and gender of emerging wasps, and the mean number of parasitoids that died before emerging were recorded per cross at each dose and host egg age.

2.3.2. Choice experiments

Parasitoid exposure was done on day 2. After incubation, the total number of eggs, the total number of parasitized eggs, the total number of emerging wasps, and the number of parasitoids that died before emerging were recorded for each FCM egg patch.

2.4. Parasitoid quality

2.4.1. No choice experiments

Parasitized egg sheets were kept at 26 ± 1 °C, a 14:10 (L:D) h photoperiod, and 65–70% RH until wasp emergence. The size of female parasitoids (as a measure of fitness) was recorded for each treatment. Female parasitoids were placed on microscope slides in a drop of water, and their size was determined by measuring the length of one hind tibia with an optical micrometer positioned in the eyepiece of a compound scope (at 40×) as suggested by Hohmann et al. (1988). Waage and Ng (1984) found that average hind tibial length predicted the egg complement in female *Trichogramma evanescens* Westwood. In total, the hind tibiae of 715 female parasitoids were measured.

2.5. Statistical analysis

Data collected from the no choice experiment were analyzed using a three-factor analysis of variance, with FCM cross, dose of radiation, and age of host eggs as sources of variation (PROC ANOVA) (SAS Institute, 1989). The first statistical model included the following dependent variables: number of parasitized eggs, number of parasitized eggs from which one or more parasitoids emerged, number and gender of emerging wasps, and number of parasitoids that died before emerging. These variables were calculated as a percentage of the total number of eggs laid and expressed as an arcsine-transformed percentage; these derived values were included in the analysis. In the second statistical model, the mean length of hind tibiae of female wasps emerging from eggs within each treatment replication was the dependent variable. When the statistical model indicated significant treatment effects and significant interactions, differences among means were separated by the Tukey–Kramer statistic ($P \leqslant 0.05$) for multiple comparisons.

Data collected from the choice experiment were analyzed using a two-factor analysis of variance, with FCM cross choice and dose of radiation as sources of variation (PROC ANOVA) (SAS Institute, 1989). The first statistical model included the following dependent variables: number of eggs in the treated and the untreated FCM crosses in the choice arena, number of parasitized eggs from the treated and the untreated FCM cross, number of parasitized eggs from which one or more parasitoids emerged for the treated and the untreated FCM cross, and number of parasitoids that died before emerging from the treated and the untreated FCM cross. In the second statistical model, the differences between the treated and the untreated FCM cross for each of the above mentioned variables were the dependent variables. When the statistical model indicated significant treatment effects and significant interactions, differences among means were separated by the Tukey–Kramer statistic ($P \leqslant 0.05$) for multiple comparisons.

3. Results

3.1. No choice experiments

The mean number of parasitized eggs was significantly influenced by the FCM host cross ($F = 8.36$; $df = 2, 98$; and $P = 0.0004$) (Table 1), dose of radiation ($F = 6.57$; $df = 2, 98$; and $P = 0.0021$) (Table 2), and age of host egg ($F = 9.59$; $df = 2, 98$; and $P = 0.0002$) (Table 3). In general, fewer eggs were parasitized when females were treated with radiation (T♀) or when the host egg age was greater than 24 h. However, there was a significant interaction ($F = 3.57$; $df = 4, 98$; and $P = 0.0093$) between dose of radiation and age of host egg in the percentage of eggs that were parasitized.

Table 1
Effect of host cross on mean (±SD) number of eggs of *C. leucotreta* that were parasitized by *T. cryptophlebiae* in no choice trials. N = untreated (fertile) adult moths and T = moths that were treated with γ-radiation (either 150 or 200 Gy)

Host cross	Mean # ± SD eggs parasitized
N♀ × T♂	25.47 ± 11.20a
T♀ × N♂	20.77 ± 12.58ab
T♀ × T♂	15.63 ± 12.68b

Means within each column followed by the same letter are not significantly different ($P > 0.05$).

Table 2
Effect of dose of radiation on mean (±SD) number of eggs of *C. leucotreta* that were parasitized by *T. cryptophlebiae* laid by host pairs where the female, the male or both members of the pair had been treated with γ-radiation

Dose of radiation (Gy)	Mean # ± SD eggs parasitized
0	25.53 ± 9.33a
150	19.17 ± 13.32b
200	17.29 ± 13.90b

Means within each column followed by the same letter are not significantly different ($P > 0.05$).

Table 3
Effect of host egg age on the mean (±SD) number of eggs of *C. leucotreta* that were parasitized by *T. cryptophlebiae*

Host egg age (h)	Mean # ± SD eggs parasitized
24	27.02 ± 11.79a
48	17.07 ± 11.27b
72	18.50 ± 12.98b

Means within each column followed by the same letter are not significantly different ($P > 0.05$).

There was a significant interaction ($F = 3.47$; $df = 4, 98$; and $P = 0.0108$) between host cross and dose of radiation with respect to the mean number of wasps emerging from each egg patch (Table 4). Fewer total wasps emerged as the dose of radiation applied to the females (T♀ × N♂ and T♀ × T♂) in the host cross increased. Similarly, the mean number of female parasitoids produced was significantly affected by an interaction between cross and dose ($F = 2.60$; $df = 4, 98$; and $P = 0.0409$) (data not shown). However, the mean number of male parasitoids was significantly influenced by the age of the host egg ($F = 5.84$; $df = 2, 98$; and $P = 0.0040$), as well as host cross ($F = 4.86$; $df = 2, 98$; and $P = 0.0097$) and dose of radiation ($F = 13.21$; $df = 2, 98$; and $P < 0.0001$) (data not shown). The mean number of parasitoids that died before emerging (Table 5) was significantly affected by an interaction between host cross and host egg age ($F = 2.92$; $df = 4, 98$; and $P = 0.0251$), as well as by an interaction between dose of radiation and host egg age ($F = 10.91$; $df = 4, 98$; and $P < 0.0001$). More parasitoids died before emerging when the host egg age was 24 h and the host cross involved irradiated females (T♀ × N♂ and T♀ × T♂). In addition, more parasitoids died before emerging when the host egg age was 24 h and the FCM pairs were treated with 150 and 200 Gy.

The mean percentage of parasitized eggs from which one or more parasitoids emerged was significantly affected by host cross ($F = 5.77$; $df = 2, 98$; and $P = 0.043$) (Table 6) and an interaction between dose of radiation and host egg age ($F = 8.48$; $df = 4, 98$; and $P < 0.0001$) (Table 7). However, the mean number of parasitized eggs from which one or more parasitoids emerged was significantly influenced by host egg age ($F = 4.25$; $df = 2, 98$; and $P = 0.0169$) and an interaction between host cross and dose of radiation ($F = 3.46$; $df = 4, 98$; and $P = 0.0108$) (data not shown). Overall, egg parasitism decreased when host eggs were laid by irradiated females (T♀ × N♂ and T♀ × T♂) and when host egg age was greater than 24 h.

The mean tibial length of female *T. cryptophlebiae* parasitoids emerging from FCM eggs was significantly affected by host cross, dose of radiation and host egg age, resulting in a significant threeway interaction ($F = 2.36$; $df = 8, 71$; and $P = 0.0262$). Significant differences between mean tibial length for female parasitoids emerging from different host crosses and doses of radiation were only detected when host eggs were 24 h-old (Table 8). Larger female parasitoids emerged from eggs that were laid by untreated females (N♀ × T♂) than

Table 4
Means (±SD) number of *T. cryptophlebiae* wasps emerging from each egg patch as influenced by the type of *C. leucotreta* host cross and the dose of radiation used to treat the female, the male or both members of the host pair. N = untreated (fertile) adult moths and T = moths that were treated with γ-radiation

Host cross	Dose of radiation			Mean (±SD)
	0 Gy	150 Gy	200 Gy	
N♀ × T♂	27.80 ± 11.05 Aa	26.00 ± 9.36 Aa	23.47 ± 13.94 Aa	25.76 ± 11.48
T♀ × N♂	27.80 ± 11.05 Aa	13.77 ± 11.05 ABab	12.00 ± 11.12 Bab	18.05 ± 13.02
T♀ × T♂	27.80 ± 11.05 Aa	7.57 ± 10.44 Bb	7.25 ± 8.92 Bb	14.88 ± 14.10
Mean (±SD)	27.80 ± 10.80	16.07 ± 12.75	14.74 ± 13.30	

Means within each row followed by the same uppercase letter are not significantly different ($P > 0.05$); means within each column followed by the same lowercase letter are not significantly different ($P > 0.05$).

Table 5
Mean (±SD) number of *T. cryptophlebiae* wasps that died before emerging as influenced by host cross, dose of radiation, and host egg age. N = untreated (fertile) adult moths and T = moths that were treated with γ-radiation.

Host egg age (h)	Host cross			Dose of Radiation			Mean (±SD)
	N♀ × T♂	T♀ × N♂	T♀ × T♂	0 Gy	150 Gy	200 Gy	
24	2.07 ± 2.69 Aa	5.27 ± 5.66 Ba	5.17 ± 5.61 Ba	0.20 ± 0.41 Aa	6.27 ± 5.64 Ba	6.25 ± 4.16 Ba	4.10 ± 4.92
48	0.60 ± 0.51 Aa	2.27 ± 3.94 Ab	1.27 ± 1.28 Ab	0.40 ± 0.51 Aa	1.80 ± 2.11 Ab	1.93 ± 3.56 Ab	1.38 ± 2.45
72	0.73 ± 1.28 Aa	1.38 ± 1.45 Ab	0.79 ± 1.37 Ab	1.60 ± 1.80 Aa	0.75 ± 0.97 Ab	0.47 ± 0.83 Ab	0.95 ± 1.36
Mean (±SD)	1.13 ± 1.83	3.05 ± 4.39	3.67 ± 3.02	0.73 ± 1.25	3.10 ± 4.30	2.64 ± 3.86	

Means within each row for each variable (Host cross, Dose of radiation) followed by the same uppercase letter are not significantly different ($P > 0.05$); means within each column followed by the same lowercase letter are not significantly different ($P > 0.05$).

Table 6
Mean (±SD) percentage of *C. leucotreta* eggs that were parasitized by *T. cryptophlebiae* from which one or more parasitoids emerged as influenced by host cross. N = untreated (fertile) adult moths and T = moths that were treated with γ-radiation (either 150 or 200 Gy)

Host cross	Mean % ± SD eggs parasitized
N♀ × T♂	25.26 ± 21.75 a
T♀ × N♂	19.97 ± 19.34 a
T♀ × T♂	15.81 ± 20.35 b

Means within each column followed by the same letter are not significantly different ($P > 0.05$).

from eggs laid by treated females (T♀ × N♂ and T♀ × T♂). Also, dose of radiation (150 or 200 Gy) resulted in the emergence of smaller parasitoid females compared with those emerging from eggs laid by untreated (0 Gy) crosses. One hundred percent of the female FCM that laid the eggs for the no choice experiments were found to have mated (a spermatophore was present in the bursa copulatrix).

3.2. Choice experiments

The number of eggs that were laid by N♀ × N♂ (control—0 Gy) crosses on one side of the mesh dome was not influenced by the type of cross (N♀ × T♂, T♀ × N♂, and T♀ × T♂) or by the dose of radiation (150 or 200 Gy) given to moth pairs on the opposite side of the mesh dome. Likewise, the number of eggs laid by the treated crosses (N♀ × T♂, T♀ × N♂, and T♀ × T♂) was not significantly influenced by the type of cross or the dose of radiation (150 or 200 Gy) given to the moths in the cross. With respect to egg patches laid by crosses involving treated insects (N♀ × T♂, T♀ × N♂, and T♀ × T♂), the total number of parasitized eggs, the total number of emerged parasitoid adults, and the number of parasitoids that died before emerging were not significantly influenced by the gender irradiated or by the dose of radiation. Similarly, with respect to egg patches laid by untreated crosses (N♀ × N♂), the total number of parasitized eggs, the total number of emerged parasitoid adults, and the number of parasitoids that died before emerging were not significantly influenced by the type of cross (N♀ × T♂, T♀ × N♂, and T♀ × T♂) or the dose (150 or 200 Gy) given to competing crosses on the opposite side of the mesh dome. The difference between the egg patches laid by crosses involving treated insects (N♀ × T♂, T♀ × N♂, and T♀ × T♂), and the control cross (N♀ × N♂) with respect to the total number of eggs per patch, the total number of parasitized eggs, the total number of emerged parasitoid adults, and the number of parasitoids that died before emerging was not significantly influenced by treatment cross or dose of irradiation. One hundred percent of the female FCM that laid the eggs for the choice experiments were found to have mated (a spermatophore was present in the bursa copulatrix).

Table 7
Mean (±SD) percentage of *C. leucotreta* eggs that were parasitized by *T. cryptophlebiae* from which one or more parasitoids emerged as influenced by host egg age and dose of radiation

Host egg age (h)	Dose of radiation			Mean (±SD)
	0 Gy	150 Gy	200 Gy	
24	60.27 ± 21.15 Aa	23.82 ± 20.49 Ba	28.23 ± 19.42 Ba	38.10 ± 26.08
48	19.56 ± 5.57 Ab	9.60 ± 11.44 Aa	12.04 ± 11.77 Aa	13.73 ± 10.68
72	13.04 ± 6.34 Ab	9.81 ± 6.03 Aa	7.45 ± 9.79 Ab	10.12 ± 7.87
Mean (±SD)	30.96 ± 24.73	14.74 ± 15.65	15.03 ± 16.02	

Means within each row followed by the same uppercase letter are not significantly different ($P > 0.05$); means within each column followed by the same lowercase letter are not significantly different ($P > 0.05$).

Table 8
Mean (±SD) tibial length in mm for *T. cryptophlebiae* parasitoids emerging from *C. leucotreta* eggs that were 24-h-old as influenced by host cross and dose of radiation. N = untreated (fertile) adult moths and T = moths that were treated with γ-radiation

Host cross	Dose of radiation			Mean (±SD)
	0 Gy	150 Gy	200 Gy	
N♀ × *T*♂	0.0140 ± 0.00023 Aa	0.0131 ± 0.00052 Aa	0.0133 ± 0.00019 Aa	0.0134 ± 0.00049
T♀ × *N*♂	0.0140 ± 0.00023 Aa	0.0120 ± 0.00060 Bab	0.0117 ± 0.00013 Bb	0.0127 ± 0.00116
T♀ × *T*♂	0.0140 ± 0.00023 Aa	0.0117 ± 0.00109 Bb	0.0108 ± 0.0 Bb	0.0127 ± 0.00147
Mean (±SD)	0.0140 ± 0.00021	0.0124 ± 0.00096	0.0125 ± 0.00102	

Means within each row followed by the same uppercase letter are not significantly different ($P > 0.05$); means within each column followed by the same lowercase letter are not significantly different ($P > 0.05$).

4. Discussion

We conducted a series of laboratory experiments to determine the acceptability and suitability of FCM eggs to parasitization by *T. cryptophlebiae* under no choice and choice experimental designs. In general, all FCM egg treatments presented to female *T. cryptophlebiae* in the no choice experiments were acceptable for oviposition and suitable for parasitoid development. Nonetheless, significant differences were detected in the number of parasitized FCM eggs when one member of the host cross, particularly the female, was treated with γ-radiation or when the age of host eggs was greater than 24 h (Tables 1–3). The mean number of FCM eggs that were parasitized was reduced by 39% when eggs were laid by treated (*T*♀ × *T*♂) crosses and by 25% when eggs were laid by *T*♀ × *N*♂ crosses as compared to parasitism in eggs laid by crosses involving untreated females (*N*♀ × *T*♂) (Table 1). In addition, when FCM were treated with 200 and 150 Gy, the number of host eggs that were parasitized was reduced by 32% and 25%, respectively, when compared with eggs laid by untreated (0 Gy) controls (Table 2).

The mean number of *T. cryptophlebiae* (Table 4), as well as the mean number of female parasitoids, emerging from FCM eggs was significantly affected by an interaction between FCM host cross and dose of radiation. The mean number of parasitoids that died before emerging (Table 5) was affected by an interaction between FCM host cross and host egg age, as well as by an interaction between dose of radiation and host egg age. Significantly more parasitoids died while developing in eggs laid by FCM treated with 150 or 200 Gy than in eggs laid by untreated (0 Gy) FCM when eggs were 24-h-old, but this difference disappeared when eggs from irradiated treatments (150 or 200 Gy) were 48- or 72-h-old (Table 5). In addition, our results showed that more *T. cryptophlebiae* died before emerging when the host egg age was 24 h and the host cross involved irradiated females (*T*♀ × *N*♂ and *T*♀ × *T*♂) (Table 5). As above, this difference disappeared as host egg age increased. It is worth noting that in the choice experiments, no differences were detected between treatments (*N*♀ × *N*♂ versus *T*♀ × *N*♂, *N*♀ × *T*♂, or *T*♀ × *T*♂) or in the doses (150 or 200 Gy) given in terms of acceptability of host eggs by *T. cryptophlebiae* as measured by the total number of eggs parasitized, the total number of emerged parasitoid adults and the number of parasitoids that died before emerging. Finally, host cross and an interaction between dose of radiation and host egg age significantly influenced the mean percentage of parasitized eggs that resulted in one or more emerged *T. cryptophlebiae* adults (Tables 6 and 7).

Female parasitoid size (as measured by the length of the hind tibia) was not significantly influenced by FCM egg age (Table 8). Nonetheless, female parasitoids emerging from eggs laid by a FCM pair where one of the members of the cross was treated with 150 or 200 Gy were smaller (i.e., had ca. 10% shorter tibiae). This might suggest a reduction in parasitoid fitness when compared with parasitoid females emerging from eggs laid by untreated FCM pairs. Hohmann et al. (1988) showed a linear correlation between female size in *T. platneri* and the number of *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) host eggs it parasitized and between parasitoid size and the number of progeny produced by a single female in her lifetime. Moreover, lifetime fecundity (as measured by egg complement 24 h after emergence when the wasps had access to honey) in *T. platneri* increased with parasitoid size. Lifetime fecundity is thought to be an important measure of a wasp's reproductive potential and thus can be used as an index of its quality as a biological control agent. Waage and Ng (1984) also reported a significant relationship between tibial length and fecundity. In addition, they noted a relationship between tibial length and longevity, and tibial length and total number of hosts parasitized. The regression equation reported by Waage and Ng (1984) for the relationship between tibial length and number of hosts parasitized suggests that a 10% reduction in the length of the hind tibiae would result in approximately 25% fewer hosts parasitized over the lifetime of a female wasp.

In practical terms, our laboratory results suggest that *T. cryptophlebiae* would accept, successfully develop in, and emerge from FCM eggs laid by the different crosses that would theoretically be produced in the field under a sterile insect release program for FCM ($N♀ \times T♂$, $T♀ \times N♂$, and $T♀ \times T♂$). Although eggs from irradiated FCM were found to be suitable as hosts, *T. cryptophlebiae* demonstrated a preference for the eggs from non-irradiated FCM. These data are congruent with studies on the acceptability of irradiated and non-irradiated codling moth eggs to *T. platneri* reported by Cossentine et al. (1996) and suggest that further field cage (Bloem et al., 1998) and field evaluations (Cossentine and Jensen, 2002) combining releases of irradiated FCM and parasitoids are warranted.

Additional control tactics that are effective and environmentally benign are needed to reduce the negative impact of FCM on citrus production in South Africa. Ideally, these control tactics should be compatible with the current augmentative biological control program using *T. cryptophlebiae*. Synergism resulting from a combined application of the sterile insect technique (SIT) and augmentative biological control has been predicted by mathematical models (Carpenter, 1993, 2000; Knipling, 1992). This approach should bring about pest suppression more rapidly than either tactic used alone. Bloem et al. (1998) found an additive effect in the control of codling moth in field cages that received a single release of sterile insects and parasitoids as compared to cages that received either treatment by itself. Additionally, Cossentine and Jensen (2002) reported that the presence of low numbers of wild and nonviable codling moth eggs in orchards under sterile (320 Gy) insect release were able to maintain low populations of *T. platneri* released twice (6–9 days apart) per season into apple orchards. Therefore, when season-long releases of sterile insects and parasitoids are made, it is anticipated that synergism of treatment effects may reduce the overall cost of the combined approach (Bloem et al., 1998).

In addition to the enhanced efficacy of a combined release strategy, additional cost saving factors may favor the application of this approach in South Africa. For example, the Cederberg Biocontrol Systems produce both FCM and *T. cryptophlebiae* parasitoids in their mass rearing facility. As such, the sharing of laboratory resources and labor reduce production costs. Also, because the Cederberg Biocontrol Systems produce an excess of FCM adults during the mass rearing of *T. cryptophlebiae*, the inclusion of FCM SIT in a control program would salvage insect material that is currently discarded, and thereby improve cost effectiveness.

The data reported herein suggest that host eggs originating from matings involving irradiated and released FCM could facilitate parasitoid population increase by supplying additional host material in the field. Furthermore, the FCM eggs not used as host material by the parasitoids would either fail to hatch or would hatch and develop into sterile F_1 adults (Bloem et al., 2003) that would provide additional FCM suppression. Currently, the SIT is under investigation as a strategy for FCM suppression in South Africa and as a tactic that could be rapidly deployed if FCM were to become established as an exotic invasive pest in other countries such as the United States. The SIT is regarded as a host-specific tactic that is environmentally friendly. However, fully successful integration of the SIT and releases of natural enemies into an effective pest management approach can occur only if the natural enemy does not negatively impact irradiated insects and their progeny more severely than it affects the feral pest population, and if the release of irradiated insects does not negatively impact the efficacy of the natural enemy (Carpenter, 1993). As such, knowledge of the compatibility of *T. cryptophlebiae* and the release of irradiated FCM is crucial to the evaluation of the combined use of these tactics. We found that the level of acceptability and suitability of eggs laid by irradiated FCM (as hosts for *T. cryptophlebiae*) was favorable for the combined use of SIT and augmentative releases of parasitoids. Based upon these results we have initiated additional studies to examine the combination of these strategies for control of FCM under field conditions.

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